

DOCKET NO: WYTH0039-101 (AM100787-D1)
APPLICATION SERIAL NO. 10/828,970
RESPONSE TO NOTICE TO COMPLY DATED OCTOBER 8, 2004
REPLY TO NOTICE TO COMPLY OF AUGUST 12, 2004

PATENT

AMENDMENTS TO THE SPECIFICATION

In the specification:

Please amend the specification after page 89 to include the Sequence Listing submitted under 37 C.F.R. §§1.821-1.825.

Please replace the paragraph bridging pages 31 and 32 with the following:

-- Estrogens are required for full ductal elongation and branching of the mammary ducts, and the subsequent development of lobulo-alveolar end buds under the influence of progesterone. In this test procedure, the mammotrophic activity of selected compounds of the invention was evaluated according to the following standard pharmacological test procedure. Twenty-eight day old Sprague-Dawley rats (Taconic Farms, Germantown, NY) were ovariectomized and rested for nine days. Animals were housed under a 12-hour light/dark cycle and fed a casein-based Purina Laboratory Rodent Diet 5K96 (Purina, Richmond, IN) and allowed free access to water. Rats were then dosed subcutaneously for six days with vehicle (50% DMSO (JT Baker, Phillipsburg, NJ) / 50% 1x Dulbecco's Phosphate buffered saline (GibcoBRL, Grand Island, NY), 17 β -estradiol (0.1 mg/kg) or test compound (20 mg/kg). For the final three days, rats were also dosed subcutaneously with progesterone (30 mg/kg). On the seventh day, rats were euthanised and a mammary fat pad excised. This fat pad was analyzed for casein kinase II mRNA as a marker of end bud proliferation. Casein kinase II mRNA was analyzed by real-time RT-PCR. Briefly, RNA was isolated following Trizol (GibcoBRL, Grand Island, NY) according to the manufacture's directions, Samples were treated with DNase I using DNA-free kit (Ambion), and casein kinase II mRNA levels were measured by real-time RT-PCR using the Taqman Gold procedure (PE Applied Biosystems). A total of 50 ng of RNA was analyzed in triplicate using casein kinase II specific primer pair (5' primer, CACACGGATGGCGCATACT (SEQ. ID NO:1); 3' primer, CTCGGGATGCACCATGAAG (SEQ. ID NO:2)) and customized probe (TAMRA-CGGCACTGGTTTCCCTCACATGCT-FAM) (SEQ. ID NO:3). Casein kinase II

DOCKET NO: WYTH0039-101 (AM100787-D1)

PATENT

APPLICATION SERIAL NO. 10/828,970

RESPONSE TO NOTICE TO COMPLY DATED OCTOBER 8, 2004

REPLY TO NOTICE TO COMPLY OF AUGUST 12, 2004

mRNA levels were normalized to 18s ribosomal RNA contained within each sample reaction using primers and probe supplied by PE Applied Biosystems. --